Neuroscience Letters 541 (2013) 49–53

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

# Melatonin induces histone hyperacetylation in the rat brain

Lennard P. Niles*, Yi Pan, Sean Kang, Ayush Lacoul

*Department of Psychiatry & Behavioural Neurosciences, McMaster University, HSC-4N77, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5*

## HIGHLIGHTS

- Novel evidence that melatonin increases histone acetylation in rat brain.
- Significant increases in histone H3 and H4 acetylation in hippocampus and striatum.
- Significant increases in phospho-ERK 1/2 observed in hippocampus and striatum.
- Findings implicate chromatin remodeling and epigenetic regulation in melatonin action.

## ARTICLE INFO

*Article history:*
Received 12 September 2012
Received in revised form 22 January 2013
Accepted 25 January 2013

*Keywords:*
Melatonin
Epigenetic
Histone acetylation
ERK 1/2
Hippocampus
Striatum

## ABSTRACT

We have reported that melatonin induces histone hyperacetylation in mouse neural stem cells, suggesting an epigenetic role for this pleiotropic hormone. To support such a role, it is necessary to demonstrate that melatonin produces similar effects in vivo. Histone acetylation, following chronic treatment with melatonin (4 μg/ml in drinking water for 17 days), was examined by western blotting in selected rat brain regions. Melatonin induced significant increases in histone H3 and histone H4 acetylation in the hippocampus. Histone H4 was also hyperacetylated in the striatum, but there were no significant changes in histone H3 acetylation in this brain region. No significant changes in the acetylation of either histone H3 or H4 were observed in the midbrain and cerebellum. An examination of kinase activation, which may be related to these changes, revealed that melatonin treatment increased the levels of phospho-ERK (extracellular signal-regulated kinase) in the hippocampus and striatum, but phospho-Akt (protein kinase B) levels were unchanged. These findings suggest that chromatin remodeling and associated changes in the epigenetic regulation of gene expression underlie the multiple physiological effects of melatonin.



The pineal indoleamine, melatonin, induces widespread physiological effects in mammals via at least two high-affinity G protein-coupled $MT_1$ and $MT_2$ receptors, which influence multiple signaling pathways through $G_i$, $G_q$, $G_s$ and other G proteins [7,10]. In addition to inhibition of adenylate cyclase (AC) activity, with a consequent decrease in cAMP signaling, melatonin has been shown to activate the mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) pathway [34,41]. Signaling studies in a model of acute ischemic stroke suggest that the acute neuroprotective effect of melatonin involves activation of the phosphatidyl inositol-3-kinase (PI-3-K)/Akt (protein kinase B) pathway, whereas ERK-1/2 and c-Jun N-terminal kinase (JNK)-1/2, in addition to Akt signaling, appear to be involved in its long-term effects [19]. There is also evidence that melatonin can interact with other cellular targets including protein kinase C, calmodulin and quinone reductase 2 [6,28]. It has been shown to potentiate a $GABA_A$ receptor-mediated current in the hypothalamus via the $MT_1$ receptor subtype, while inhibiting this current in the hippocampus through the $MT_2$ subtype [40]. Since both of the melatonin receptor subtypes are linked to inhibition of cAMP production, the mechanisms generating the differences in $GABA_A$ receptor responses presumably involve other divergent signaling pathways. In accordance with this, transfection studies with human melatonin receptors in HEK cells indicate that the $MT_2$, but not the $MT_1$, receptor is coupled to inhibition of the cGMP pathway [30]. Thus, melatonin can interact with multiple cellular targets to produce its diverse effects [13]. Moreover, recent studies indicate that physiological concentrations of melatonin increase histone H3 acetylation in mouse C17.2 neural stem cells, suggesting a role in epigenetic regulation for this hormone [37]. In order to determine the potential significance of this in vitro observation, we have examined whether melatonin induces similar modifications of histone H3 and H4 proteins in vivo.

Male Sprague-Dawley rats (3–4 weeks old) were maintained under a 12:12 lighting cycle with lights on at 07:00 h and allowed to acclimatize for one week before the start of treatment. Animals were randomly assigned to control or melatonin treatment groups and they had free access to food and water. Control animals

* Corresponding author. Tel.: +1 905 525 9140x22224; fax: +1 905 522 8804.
*E-mail addresses:* niles@mcmaster.ca (L.P. Niles), yipan2010@hotmail.com (Y. Pan), wolverine_007_@hotmail.com (S. Kang), ayushlacoul@hotmail.com (A. Lacoul).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.01.050

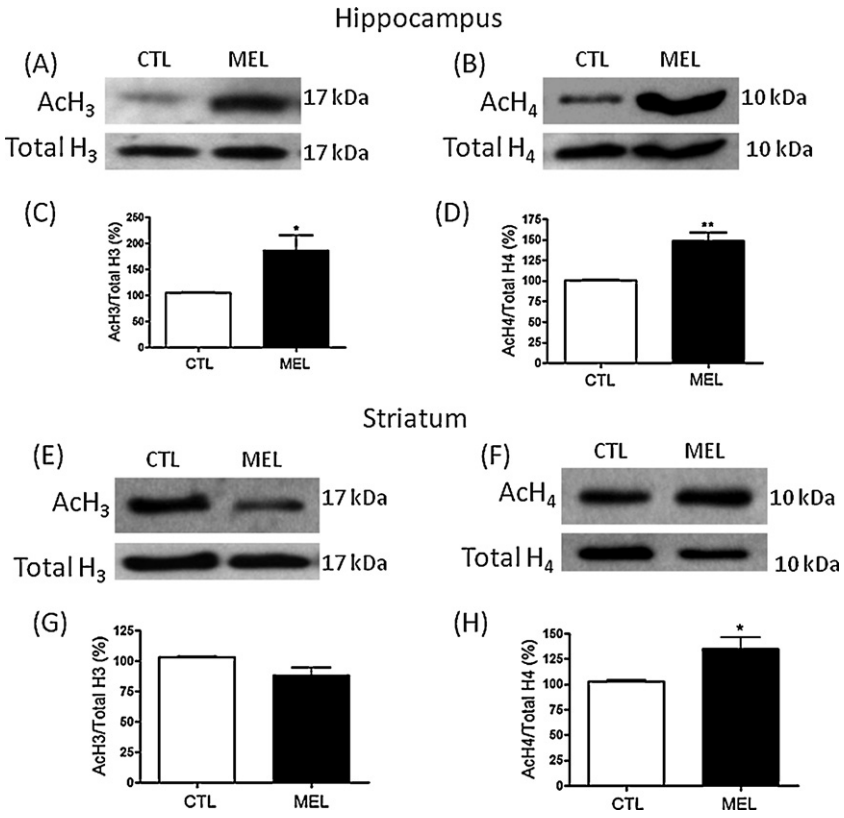


**Fig. 1.** Hyperacetylation of histone H3 and/or histone H4 in rat hippocampus and striatum following chronic melatonin treatment. Representative immunoblots of acetylated H3 (AcH3), acetylated H4 (AcH4), total H3 and total H4 histones in the hippocampus (A, B) and striatum (E, F), as indicated. Quantification of AcH3 and AcH4 in hippocampus (C, D) and striatum (G, H), as a percentage of total H3 and total H4, respectively. Data shown are means ± S.E.M. ($n = 3$). $^{*}p < 0.05$; $^{**}p < 0.01$ vs control.

received the melatonin vehicle (0.04% ethanol in drinking water) and the treatment group received melatonin (4 μg/ml) in their drinking water, as previously reported [36]. After 17 days, animals were decapitated and the entire hippocampus, both striata, midbrain and cerebellum were rapidly dissected on ice, using an established procedure [15]. Brain tissues were snap-frozen on dry ice/ethanol and stored at −80 °C, until used for protein analysis. All experiments were carried out according to the guidelines set by the McMaster University Animal Research Ethics Board.

Brain tissue samples were homogenized in ice-cold buffer (pH 7.4) containing 10 mM Tris–HCl, 5 mM NaF, 1 mM $Na_3VO_4$, 1 mM EDTA, 1 mM EGTA and 320 mM sucrose. The homogenates were incubated on ice for 10 min and then centrifuged ($800 \times g$) for 10 min at 4 °C. Following removal of the cytosolic fraction, the nuclear pellet was resuspended in 500 μL of 0.4 N $H_2SO_4$ and incubated on ice for 30 min. The supernatant containing nuclear protein was collected by centrifugation ($14{,}000 \times g$) for 10 min at 4 °C and transferred to a fresh tube. Nuclear proteins were precipitated with 100% trichloroacetic acid containing 4 mg/mL deoxycholic acid. The pellet was washed with ice-cold acetone and resuspended in 10 mM Tris–HCl (pH 8.0). Protein concentrations were determined with the DC Protein Assay Kit (Bio-Rad Laboratories, Mississauga, ON).

For the assessment of histone acetylation, 10 μg of nuclear proteins were electrophoresed on 15% sodium-docecyl-sulfate polyacrylamide gels (SDS-PAGE) for approximately 1 h at 200 V (4 °C). To detect the phosphorylation of ERK1/2 and Akt, 20 and 60 μg (respectively) of cytoplasmic proteins were separated on the gel. The proteins were then transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore Corporation, Billerica, MA) overnight at 25 V (4 °C). Membranes were blocked with 5% skim milk in Tris Buffered Saline (TBS) for 1 h and then incubated with the following primary antibodies, at the indicated dilutions: acetyl-histone H3 (K9/18) (1:2500; EMD Millipore Corporation, Billerica, MA), histone H4 pan-acetyl antibody (1:5000; Active Motif, Carlsbad, CA), phospho-p44/42 (ERK1/2) (Thr202/Tyr204) (1:2000) and phospho-Akt (Ser473) (1:2000 dilution; Cell Signaling Technology, Danvers, MA). Subsequently, membranes were stripped and re-probed with primary antibodies against total histone H3 (1:5000), total histone H4 (1:5000; Active Motif, Carlsbad, CA), ERK1/2 (p44/p42) clone MK12 (1:5000; EMD Millipore Corporation, Billerica, MA), and Akt (1:1000; Cell Signaling Technology, Danvers, MA). Following incubation with primary antibodies, membranes were incubated with an HRP- conjugated goat anti-rabbit IgG (histone H3, histone H4, Akt) or a goat anti-mouse IgG (ERK1/2) in TBS with 5% skim milk for 2 h at room temperature. Following treatment with enhanced chemiluminescence (ECL) reagents (Amersham Pharmacia Biotech, Piscataway, NJ), proteins were detected by film fluorography and optical density analysis performed with AlphaImage 2200 software. Western data for acetylated or phosphorylated proteins were normalized with respect to total histone H3, total histone H4, total ERK1/2 or total Akt. After conversion to percentage values, data were analyzed by unpaired Student's $t$ test, with $p \leq 0.05$ taken as the level of significance. Data shown are means ± S.E.M.

There were no differences in either body weight (control: 290 ± 6 g, $n = 4$; melatonin: 289 ± 10 g, $n = 5$) or weekly water consumption (control: 223 ± 13 ml, $n = 4$; melatonin: 240 ± 12 ml, $n = 5$). Chronic treatment with melatonin caused a significant increase in histone H3 (Fig. 1A and C; $p < 0.05$) and histone H4 (Fig. 1B and D; $p < 0.01$) acetylation in the hippocampus. Similarly, histone H4 acetylation was increased ($p < 0.05$) in the striatum (Fig. 1F and H), but there was no significant change in histone H3